Europäisches Patentamt
European Patent Office
Office européen des brevets

Bescheinigung Certificate Attestation

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. Patent application No. Demande de brevet n°

04250508.1

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

Anmeldung Nr:
Application no.: 04250508.1
Demande no:

Anmeldetag:
Date of filing: 30.01.04
Date de dépôt:

Anmelder/Applicant(s)/Demandeur(s):

Insense Limited
Colworth House
Sharnbrook,
Bedford MK44 1LQ
GRANDE BRETAGNE

Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

Improvements in or relating to skin dressings

In Anspruch genommene Priorität(en) / Priority(ies) claimed /Priorité(s) revendiquée(s)
Staat/Tag/Aktenzeichen/State/Date/File no./Pays/Date/Numéro de dépôt:

Internationale Patentklassifikation/International Patent Classification/
Classification internationale des brevets:

A61L15/00

Am Anmeldetag benannte Vertragstaaten/Contracting states designated at date of filing/Etats contractants désignées lors du dépôt:

AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LU MC NL PT RO SE SI SK TR LI

C426.01/I

Title: Improvements in or relating to skin dressings

Field of the Invention

This invention relates to skin dressings for application to a part of a human or animal body for treatment of skin, and relates particularly (but not exclusively) to wound dressings for treatment of compromised skin, particularly skin lesions, i.e. any interruption in the surface of the skin, whether caused by injury or disease, including skin ulcers, burns, cuts, punctures, lacerations, blunt traumas, acne lesions, boils etc.

Background to the Invention

WO 03/090800 discloses a skin dressing comprising oxidoreductase enzyme, e.g. glucose oxidase, in a hydrated hydrogel, e.g. of hydrophilic polymer material, with one preferred polymer being poly 2-acrylamido-2-methylpropane sulphonic acid (poly-AMPS) or salts thereof (e.g. as described in WO 01/96422). The dressing may include a source of substrate for the oxidoreductase enzyme, β-D glucose in the case of glucose oxidase. For example, Figure 6 of WO 03/090800 discloses a skin dressing comprising a lower, skin-contacting layer including 20% by weight sodium poly-AMPS and 20% by weight glucose (substrate), and an upper layer in the form of a film of polyvinyl alcohol (PVA) incorporating glucose oxidase (enzyme).

The dressings of WO 03/090800 are used by being located on the skin of a human or animal, e.g. over a wound or on a region of skin to be treated for cosmetic or therapeutic purposes, e.g. for treatment of acne or other skin conditions. The oxidoreductase enzyme catalyses a reaction of an appropriate substrate with oxygen to produce hydrogen peroxide in a controlled manner in the dressing. The hydrogen peroxide diffuses through the dressing to the dressing/skin interface, where it has beneficial effects, e.g. being converted to oxygen by the enzyme catalase which is naturally present in wounds. Oxygen produced

in this location inhibits anaerobic bacteria and supports the essential metabolism of cells engaged in the healing process.

We have now found that the profile of oxygen concentration within the space under the dressing (equivalent to the wound bed) follows a predictable profile, starting with a period of oxygen depletion, caused by the freshly placed dressing blocking the supply of atmospheric oxygen. This is followed by a sustained rise in oxygen, as the dressing starts to transmit oxygen via the diffusion of hydrogen peroxide, generated in-situ. Subsequently, the oxygen level reaches a plateau at saturation, and in the longer term (depending on the stage of the wound), gradually declines.

We have also found, surprisingly, that the time course of this profile varies greatly in proportion to the concentration of polymer within the gel, even though all other (active) ingredients remain the same (other than water, of course). The discovery of this effect now allows us to design dressings in which we can control the oxygen delivery profile to match the needs of a wound bed.

In particular, we have found that it is beneficial to use a hydrated hydrogel with a higher concentration of polymer material and a lower concentration of water than those specifically disclosed in WO 03/090800 for the enzyme-containing gel at least, and preferably also for a separate substrate-containing gel.

Summary of the Invention

The present invention thus provides a skin dressing comprising oxidoreductase enzyme in hydrated condition in a hydrated hydrogel of hydrophilic polymer material, wherein hydrogel comprises at least 25% by weight of the polymer material.

The dressing preferably comprises a separate, second, hydrated hydrogel of hydrophilic polymer material containing a source of substrate for the oxidoreductase enzyme, the hydrogel comprising at least 25% by weight of the polymer material.

The or each hydrogel preferably comprises at least 30% by weight of the polymer material, and may comprise higher amounts, e.g. at least 40% by weight of the polymer material.

The polymer material preferably comprises poly-AMPS or salts thereof.

The dressing preferably comprises a lower, skin-contacting layer including substrate and an upper layer including enzyme.

Particularly good results have been obtained with an enzyme-containing hydrogel comprising 15% by weight sodium poly-AMPS and 15% by weight ammonium poly-AMPS, and a substrate-containing hydrogel comprising 30% by weight sodium poly-AMPS.

The dressing may otherwise be generally as disclosed in WO 03/090800 for most dressings of this type.

The currently preferred enzyme is glucose oxidase, with the corresponding substrate being glucose. Glucose is conveniently present in lower concentration than envisaged in WO 03/090800, e.g. constituting 5% by weight of the associated hydrogel: it has been found that greater amounts are superfluous and unnecessary.

A currently preferred dressing in accordance with the invention thus comprises a lower, skin-contacting layer comprising a hydrated hydrogel comprising 30% by weight sodium poly-AMPS and 5% by weight glucose, and an upper layer comprising a hydrated hydrogel comprising 15% by weight sodium poly-AMPS, 15% by weight ammonium poly-AMPS, and glucose oxidase.

Using hydrogels with a higher concentration of polymer material is found to affect the rate of internal hydrogen peroxide production and hence the oxygen concentration profile

beneath the dressing in use in a manner beneficial to wound healing. In particular it results in an initial period of hypoxia (absence of oxygen) after location of the dressing on a surface that, surprisingly, is beneficial. We believe that, in general, there can be a need to have a period of hypoxia, during which time cells are stimulated to produce a cytokine called "hypoxia induced factor" (HIF). This triggers a cascade of cell signalling and biochemical responses that combine to bring about the process of neovascularisation, i.e. the formation of new blood vessels, central to the healing process. HIF production is considered to be crucial to the whole healing process, and our investigations have shown that a suitably prolonged period of hypoxia is beneficial.

The initial period of hypoxia is followed by a phase of oxygen generation at the interface between wound and dressing, resulting in an oxygen surge, until a saturated oxygen concentration is reached and maintained for a period of time. This is beneficial for wound healing. In particular, it is understood that wounds benefit by experiencing a period (or periods) of high oxygen concentration, to accelerate cell metabolism, provide white blood cells with high oxygen levels through which to enhance their antimicrobial biochemistry (respiratory burst) and to inhibit or eliminate pathogenic anaerobic bacteria.

Finally, it is also clear that a saturated oxygen concentration should not be maintained indefinitely, so any system of oxygenation should provide a longer term steady state (over days) of relatively low oxygen supply, or otherwise be readily controllable. This is inevitably achieved with a dressing in accordance with the invention, either by its tendency to suppress itself while in a dry state, or by its steady swelling (and dilution) on contact with an exuding wound, or through the simple process of being deliberately changed by the user at appropriate times. In this latter case, the patient or carer can control oxygen delivery to the wound by utilising the predictable delivery profile of the dressing to intervene at defined time points, so as to tailor an oxygen delivery profile according to a treatment plan. This exploits the single-use, disposable nature of the dressing of this invention.

The present invention is based on the following observations and conclusions:

- The concentration of the polymer, e.g. poly-AMPS, has a considerable effect on the rate of changes in oxygen concentration beneath the dressing, thus permitting dressings to be designed to deliver different oxygen profiles, according to the needs of different wounds.
- The duration of the initial period of hypoxia following the application of the dressing increases with increasing concentration of polymer, e.g. poly-AMPS, in the dressing.
- The rate of subsequent increase in oxygen concentration beneath the dressing is indirectly proportional to the polymer, e.g. poly-AMPS, concentration. The time required to achieve complete oxygenation (i.e. dissolved oxygen concentration equivalent to a solution equilibrated with pure gaseous oxygen) beneath the dressing is thus longer when using a high concentrated poly-AMPS dressing than when using lower concentration poly-AMPS dressings.

The overall conclusion is that there is in general considerable benefit in using a dressing incorporating one or more hydrogels comprising about 30% by weight poly-AMPS or salts thereof as the initial period of hypoxia in use of the dressings is extended to a highly advantageous degree.

Further beneficial effects of use of higher polymer, lower water content hydrogels are that the gels are more robust and easier to handle, and also retain structural integrity over time and so are less likely to leave debris at a wound site after use. The hydrogels also have higher water absorption properties.

The invention will be further described, by way of illustrations, in the following examples and with reference to the accompanying drawings in which:

Figure 1 is a graph of % of dissolved oxygen concentration with respect to air saturated solution at 25°C versus time (in minutes), showing the rate of oxygenation of a hydrogel/sensor interface as a function of poly-AMPS concentration.

EXAMPLES

Example 1

Experiments were carried out using the following materials:

Sodium AMPS - Lubrizol, code 2405
Glucose - Fisher - analytical grade, code G050061
Potassium iodide - Fisher - analytical grade, code P584050
1-hydroxy cyclo hexyl phenyl ketone (99%) - Aldrich - 40,561-2 (this substance is referred to as 'photoinitiator')
Ebecryl 11 (PEG 400 diacrylate) - UCB Chemicals (this substance is referred to as 'cross-linker')
Glucose Oxidase - Biocatalysts - G638P (about 70kU/gram powder)
Zinc L-lactate, hydrate - Aldrich

Gel Preparation

The components were mixed in the combinations and quantities set out in Table 1, following the basic procedure set out below.

Stock solutions (as supplied by the manufacturer) of sodium AMPS were dispensed into a 250 ml polypropylene, screw-top reaction jar as the basis of the pre-gel fluid. Glucose oxidase (in the case of the top gel) and glucose, potassium iodide and zinc L-lactate (in the case of the lower or base gel) were added to the mixture and allowed to dissolve completely. In a separate vessel the photoinitiator powder was dispersed in the liquid cross-linker and the mixture was warmed gently to dissolve the photoinitiator into the cross-linker. This solution was then mixed into the pre-gel fluid. To cast the gels, the complete pre-gel fluid was poured into a flat bottomed tray, to a depth of 1-2 mm. The gels were set by UV irradiation from a 1 kW lamp, at a vertical distance of 15 cm, for 25 seconds. The gels were allowed to cool before use.

Table 1. Composition of hydrogels used in the study.

Component	Concentration of the stock solution (w/w)	Concentration in the final gel (w/w)
Components used in the top (enzyme) gels		
Na AMPS	50% aq	20% or 30% or 40%
Cross-linker	undiluted	0.20%
Photoinitiator	undiluted	0.01%
Glucose oxidase	solid powder	90 μg/g
Water		to total weight
Components used in the base gel		
Na AMPS	50% aq	20% or 30% or 40%
Glucose	solid powder	20%
Potassium iodide	10% aq	0.05%
Zinc L-lactate	5% aq	0.1%

Active oxygenation monitoring

A chronoamperometric technique using specially modified screen-printed sensors was adopted to monitor the concentration of dissolved oxygen. Sensors were printed on an alumina substrate. Carbon paste (ED5000 from Electra Ltd, UK) was used to print the working electrode, the counter electrode and the connector tracks; Ag-AgCl paste was used to print the reference electrode. The working area of the sensors was covered tightly with a 0.005'' (0.013 mm) Teflon (Teflon is a Trade Mark) layer (Fluorocarbon) with the inner electrolyte (sodium phosphate, pH 6, 0.1 M; containing KCl, 0.1M) entrapped between the sensor surface and the Teflon layer.

The principle of the technique was identical to that of the commercially available 'Clark oxygen sensors'. Dissolved oxygen diffuses through the Teflon layer into the electrode electrolyte where it is reduced at a working electrode poised at –550 mV vs. the Ag-AgCl reference electrode. The resulting cathodic current is proportional to concentration of dissolved oxygen.

Active oxygenation was monitored at the hydrogel/sensor interface. This was to mimic the processes occurring in vivo at the wound/dressing interface. A piece (approximately 2.5 × 2.5 cm) of the base gel layer was placed onto the surface of the sensor. 20 μL of electrode buffer containing catalase (100 μg mL^{-1}) was placed between the sensor and the base layer. The system was activated by placing a piece (approximately 1.5 × 1.5 cm) of the enzyme gel layer onto the base layer and dissolved oxygen concentrations were monitored beneath the base layer (i.e. at the hydrogel/sensor interface).

Results and Discussion

Three stages of oxygen concentration profile were observed at the sensor/hydrogel interface following application of the freshly activated hydrogel dressing onto the sensor. The system was activated at time 0 by bringing the two hydrogel layers together. Saturation of the electrochemical response in the region 250-300% on the y-axis corresponds to reaching the maximum oxygen solubility. This is in accord both with calibration data and with visual evidence: gas started evolving at the gel/sensor interface when the oxygenation values stabilised at the 250-300% mark on the y-axis. The poly-AMPS concentrations stated refer to both the base layer and the top layer. Glucose concentration in the base layer was 20% w/w and glucose oxidase concentration in the enzyme layer was 90 μg per gram of gel.

Results are shown in Figure 1.

As shown in Figure 1, the three stages of oxygen concentration profile are as follows:

1) First, there was a gradual decline in dissolved oxygen concentration reflecting the low solubility of oxygen in the hydrogel. The sensor interface was 'suffocated' by the hydrogel.

2) After some time (20-90 minutes depending on concentration of poly-AMPS) the concentration of oxygen started increasing. This was due to the delivery of hydrogen

proxide to the dressing/sensor interface and its immediate breakdown to oxygen by the enzyme catalase.

3) Finally, when the concentration of the dissolved oxygen at the dressing/sensor interface reached saturation the electrochemical signal stabilised. Slow evolution of gas was observed at the dressing/sensor interface shortly after.

The time profile of the above processes observed at the dressing/sensor interface was found to be dependent upon the concentration of poly-AMPS in the hydrogels.

The duration of the initial decline in oxygen concentration ('sensor suffocation') increased with increasing concentration of poly-AMPS. This was due to the slower generation of hydrogen peroxide in the top hydrogel layer and subsequent slower diffusion of peroxide to the sensor interface. The time required for the oxygen delivery to start at the interface was thus longer with a more concentrated poly-AMPS hydrogel than with that using a less concentrated poly-AMPS hydrogel.

The subsequent rate of the increase of oxygen concentration was higher in the case of low poly-AMPS concentration than with higher concentrations. This was also caused by more rapid generation of peroxide in the top gel and its more rapid diffusion towards the sensor interface. Complete oxygenation (i.e. dissolved oxygen concentration equivalent to a solution equilibrated with pure gaseous oxygen) could be achieved at the dressing/sensor interface in approximately 50 minutes following application of the top gel layer using 20% poly-AMPS, in approximately 300 minutes using 30% poly-AMPS and in approximately 500 minutes using 40% poly-AMPS (Fig.1).

Example 2

The ensuing composition in accordance with the invention is a skin treatment product of the form shown in Figure 6 of WO 03/090800, which comprises a glucose-containing hydrogel slab as a lower layer of the product, and an upper layer comprising a poly-AMPS hydrogel that incorporates glucose oxidase.

The hydrogel lower layer was formulated to include the following ingredients by weight:

Water (ex Fisher, distilled, de-ionised, analytical grade)	64.7%
Sodium AMPS (ex Lubrizol AMPS 2405 Monomer)	30.0%
Polyethylene glycol diacrylate (PEG400 diacrylate, ex UCB Chemicals available as Ebecryl 11)	0.19%
1-hydroxycyclohexyl phenyl ketone (a photoinitiator, ex Aldrich)	0.01%
Anhydrous glucose (enzyme substrate, ex Fisher)	5.00%
Potassium iodide (ex Fisher)	0.05%
Zinc L-lactate hydrate (ex Aldrich)	0.10%

The mixture was dispensed into casting trays containing either polyester scrim (polyester non-woven, open mesh support, available from HDK Industries Inc, Product Code 5722) or polyethylene net support, of dimensions 100mm x 100mm, to a depth of about 1.5mm. The polyethylene net support was fabricated from polyester staple fibres thermally bonded by a polyester resin - Product code 5722, from Castle Industries, Greenville, SC 9609, USA. The hydrogel was then set, by irradiation under a UV lamp, for up to 60 seconds and a power rating of approximately 100mW/cm^2. The hydrogel was then allowed to cool to 30°C or below.

The enzyme-containing hydrogel was formulated to include the following ingredients by weight:

Water (ex Fisher, distilled, de-ionised, analytical grade)	68.6%
Sodium AMPS (ex Lubrizol AMPS 2405 Monomer)	15.0%
Ammonium AMPS (ex Lubrizol AMPS 2411 Monomer)	15.0%
Polyethylene glycol diacrylate (PEG400 diacrylate, ex UCB Chemicals available as Ebecryl 11)	0.19%
1-hydroxycyclohexyl phenyl ketone (a photoinitiator, ex Aldrich)	0.01%
Glucose oxidase (GOX, Biocatalysts, Pontypridd, Code G575P)	0.035%

Zinc L-lactate hydrate (ex Aldrich)	1.0%
Pluronic P65 (block co-polymer of ethylene oxide and propylene oxide, HO-[CH2CH2O]x-[CH2CHCH3O]y-[CH2CH2O]y-H, average MW 3400 (BASF)	0.15%

The mixture was dispensed into casting trays containing polyester scrim (polyester non-woven, open mesh support, available from HDK Industries Inc, Product Code 5722) of dimensions 100mm x 100mm, to a depth of about 1.0mm. The hydrogel was then set, by irradiation under a UV lamp, for up to 30 seconds (typically 25 seconds), and a power rating of approximately 100mW/cm^2. The hydrogel was then allowed to cool to 30°C or below.

The enzyme-containing hydrogel and the glucose-containing hydrogel were bought together, one overlying the other.

An oxygen-permeable and moisture-permeable covering or overlay such as of polyurethane may be located over the enzyme-containing hydrogel and may be adhered to the skin by means of e.g. acrylic adhesive provided on the lower face of the overlay.

The resulting product was packaged in an oxygen-impermeable pouch or enclosure, e.g. made of laminated aluminium foil pouches as supplied by Sigma (code Z183407).

CLAIMS

1. A skin dressing comprising oxidoreductase enzyme in hydrated condition in a hydrated hydrogel of hydrophilic polymer material, wherein hydrogel comprises at least 25% by weight of the polymer material.

2. A dressing according to claim 1, further including a separate, second, hydrated hydrogel of hydrophilic polymer material containing a source of substrate for the oxidoreductase enzyme, the hydrogel comprising at least 25% by weight of the polymer material.

3. A dressing according to claim 1 or 2, wherein the or each hydrogel comprises at least 30% by weight of the polymer material.

4. A dressing according to any one of the preceding claims, wherein the polymer material comprises poly-AMPS or salts thereof.

5. A dressing according to any one of the preceding claims, comprising a lower, skin-contacting layer including substrate and an upper layer including enzyme.

6. A dressing according to claim 5, comprising an enzyme-containing hydrogel comprising 15% by weight sodium poly-AMPS and 15% by weight ammonium poly-AMPS, and a substrate-containing hydrogel comprising 30% by weight sodium poly-AMPS.

7. A dressing according to any of the preceding claims, wherein the enzyme is glucose oxidase.

8. A dressing according to claim 7 when dependent on claim 2, wherein the substrate is glucose.

9. A dressing according to claim 8, wherein glucose constitutes 5% by weight of the second hydrogel.

10. A dressing according to any of the preceding claims, comprising a lower, skin-contacting layer comprising a hydrated hydrogel comprising 30% by weight sodium poly-AMPS and 5% by weight glucose, and an upper layer comprising a hydrated hydrogel comprising 15% by weight sodium poly-AMPS, 15% by weight ammonium poly-AMPS, and glucose oxidase.

C426.01/I

ABSTRACT

Title: Improvements in or relating to skin dressings

A skin dressing of the general form disclosed in WO 03/030800 comprises oxidoreductase enzyme in hydrated condition in a hydrated hydrogel of hydrophilic polymer material, wherein hydrogel comprises at least 25% by weight of the polymer material. A currently preferred dressing comprises a lower, skin-contacting layer comprising a hydrated hydrogel comprising 30% by weight sodium poly-AMPS and 5% by weight glucose, and an upper layer comprising a hydrated hydrogel comprising 15% by weight sodium poly-AMPS, 15% by weight ammonium poly-AMPS, and glucose oxidase. Using hydrogels with a higher concentration of polymer material is found to affect the rate of oxygen generation and hence the oxygen concentration profile beneath the dressing in use in a manner beneficial to wound healing.

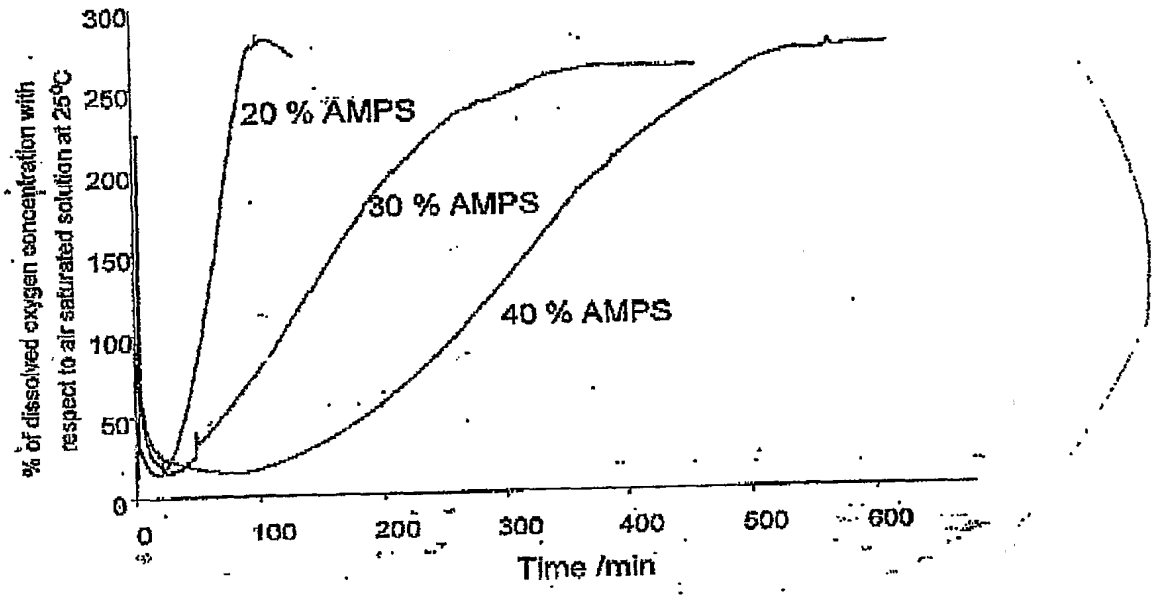

FIG 1